IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Fabrizio Samaritani, et al.
Serial No. :08/737,633
Filed: : November 15, 1996
For: IFN-BETA LIQUID FORMULATIONS

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GroupArt Unit: 1646
Examiner: D. Fiztgerald

considered
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DECLARATION

I, Dr. Pierandrea Esposito, born in Ascoli Piceno (Italy), May 10th, 1961, do solemny and sincerely declare as follows:

1. I am an Employee of Istituto di Ricerca Cesare Serono, part of Serono Group, since March 15th, 1998, holding the position of Director of the Drug Delivery Systems Unit, based in Ivrea (Turin, Italy)
2. I obtained a University Degree (recognized by EU) in Pharmaceutical Chemistry and Technology from the University of Trieste (Italy), on July 24th, 1987
3. I am a licensed pharmacist (Italian State Exam) , since November 1987
4. I have worked in the pharmaceutical industry for 10 years, holding positions up to Leader of the Drug Delivery Systems Development group, with Vectorpharma SpA, Trieste, a company specialized in research and development of innovative delivery systems and formulations, and with IRCS-Serono, which I joined in March 1998. During my activities in Vectorpharma I spent almost four years as a visiting scientist at the College of Pharmacy, University of Kentucky, USA, collaborating with the groups of Prof. George Digenis (evaluation of solid and colloidal dosage forms in vivo by gamma scintigraphy) and of Prof. Patrick De Luca (formulation development of peptide drugs in polymeric microparticles). I published 10 full papers in internationally recognized journals, and 32 abstracts in international journals and conferences ; I am coinventor in 5 patents assigned to or applied by Vectorpharma; I have been invited as a lecturer to several international Conferences, and as a teacher to the European Continuing Education College; I am also a coadvisor in university theses at the Department of Biophysics and Macromolecular Chemistry at the University of Trieste. My experience in these companies and during my studies involved extensive and specific work on the physico chemical characterization, preformulation, formulation and stabilisation of pharmaceutical compounds.

5. I have read and understood the US Patent Application Serial No.08/737,633 and the References cited by the Examiner, in particular, US Patent 5,004,605 ("Hershenson").

6. On the basis of my knowledge and of the study of the references cited by the examiner, I am convinced that US Pat. Application No. 08/737,633 brings some clear element of novelty, not expectable on the basis of the prior art knowledge.

More specifically, no prior art teaching clearly states that recombinant beta-interferon can be stabilized in a liquid formulation by the use of *mannitol alone*, or optionally in combination with albumin . The cited Hershenson's teaching (US Pat. 5,004,605) claims that recombinant β interferon can be stabilized by the specific use of polyols such as polyethylen glycol or glycerol, at a pH of 2 to 4, preferrably obtained by phosphate buffer. It is further mentioned that the invention "...can further comprise an *additional* stabilizing agent, such as a carbohydrate, for example ... mannitol... or human serum albumin, HSA, which can be used alone or in combination with a carbohydrate stabilizing agent...". Now, the generally known fact in the art that some carbohydrates, such as mannitol, are used as stabilizing agents in various formulation, does not necessarily imply that they can explicate a stabilizing effect on *any specific compound, unless clearly stated and demonstrated.* By the same reasoning, Hershennson's invention is based on the generally known fact that polyethylene glycols and glycerol are commonly used, chemically stable vehicles in the liquid formulation of stable, parenteral dosage forms (Handbook of Pharmaceutical Excipients, American Pharm. Assoc., 1st edition, (1986), respectively pgg. 209-216 and 123-124); therefore it could not be patentable unless a specific, unexpected effect was claimed.

Hershenson fails to demonstrate that a stabilizing effect on r -β IFN can be obtained by the use of mannitol and or albumin alone or in combination. Furthermore in my opinion, it cannot be considered obvious that mannitol can stabilize interferon, or any other protein, only on the basis of its general use as "stabilizer" . US Pat. Appl. 08/737,633 clearly states that unexpectedly the use of *mannitol alone*, at a specific, relatively narrow pH (3 to 4), preferrably obtained by acetate buffer, brings to a stable, liquid formulation of r -β IFN. This is not an obvious finding, and it is demonstrated by the data provided. To stress the point, it can be noticed in the detailed description of the invention (see for example Table 4 and 5) , that not all the compounds commonly used in the art as "stabilizers" (i.e. mannitol, sucrose, glycine), sort the *same* effect on r -β IFN stabilization. Therefore, *no "a priori" prediction* can be made on the stabilizing effects on a specific protein, on the basis of

a general use as stabilizer; and no assumption of this effect can be made for a compound unless specifically demonstrated.

Another innovative element of the invention resides in the finding that the use of acetic acid/acetate buffer brought unexpected stabilization to the formulaton, when compared to other buffers (i.e. citrate, ascorbate, succinate). Such stabilization effect cannot be attributed only to the pH range obtained, but also to the specific chemical structure of the buffer. Therefore, the invention of Us Pat. Appl. 08/737,633 maintains its degree of novelty also in the choice of one or more appropriate buffers, which, in a defined, carefully chosen pH range (see Table 1-3) and *in combination with mannitol* (and human serum albumin), yield to a stable formulation for r -β IFN.

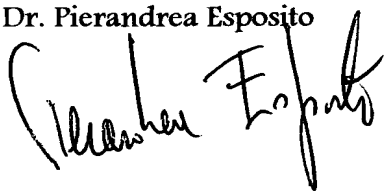
In conclusion, I believe that the present patent application retains at least *two specific aspects of innovation*, related to the specific stabilization effect of mannitol (possibly also in the presence of Human Serum Albumin) on r -β IFN, in the presence on an appropritely chosen buffer systems, yieling to a defined and narrow pH range, peaking at pH 3.5.

I further declare that all statements made therein of my own knowledge are true and that all the statements made on information and belief are believed to be true and further that these statements made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under '1001 Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date : August 19th, 1998 ,
Ivrea (Turin) , Italy

Signature

Dr. Pierandrea Esposito



IFN-β LIQUID FORMULATIONS

The present invention relates to liquid formulations of interferon-beta (IFN-β) stabilised with a polyol, a non-reducing sugar or an amino acid. In particular, it relates to liquid formulations containing mannitol, human albumin and acetate buffer.

Interferons (alpha, beta, gamma) are glycoproteins produced in the cells of vertebrates following induction. The most traditional inducers are virus, but also other microbial agents, other natural substances and synthetic compounds have the same behaviour.

Interferon-ß is induced in human fibroblasts, has anti-viral activity, but in the therapy of some tumoral forms, other activities can be exploited together with the anti-viral, such as the anti-proliferative cellular activity and immunoregulatory activity.

Production from culture of human fibroblasts, and specifically from recombinant DNA techniques, now allows to obtain industrial quantities of interferon-beta.

It is known that proteins in the purified form are especially susceptible to degradation, even due to the normal activity of atmospheric agents. This peculiarity becomes even more evident for proteins produced according to recombinant DNA techniques.

As a direct consequence of the fact that highly purified proteins are easily subject to denaturization, it becomes desirable to obtain stable formulations which ensure the longest possible life-cycle to the product.

Stabilisation of formulations containing highly purified proteins may be carried out by the addition of one or more excipients which inhibit or delay degradation of the active principle.

Pharmaceutical compositions containing interferon-beta are well known. EP Patent application 89 245 (INTER-YEDA Ltd) describes a lyophilised composition of interferon-beta containing mannitol, human albumin and polyvinylpyrrolidone, the latter as stabilising agent. Also known are pharmaceutical liquid compositions containing other interferons.

International Patent Application WO 89/04177 (GENENTECH - Priority 03/11/87) describes liquid pharmaceutical formulations of gamma-

interferon comprising a buffer which maintains the pH within the range of 4.0-6.0. a polyhydroxylate sugar as stabiliser and a non-ionic detergent.

EP Patent Application 270 799 describes IFN-β pharmaceutical compositions in liquid form or lyophilized, which comprise, as solubilizer/stabilizer, one or more non-ionic polymeric detergents.

It is highly desirable to obtain such liquid formulations in order to avoid the reconstitution of lyophilised preparations and thus to permit ease of use.

It has now surprisingly been found that liquid pharmaceutical formulations comprising interferon-beta stabilised with a polyol, a non-reducing sugar or an amino acid in an appropriate buffer result particularly stable and maintain biological activity for a long period of time.

The main object of the present invention is to provide a liquid pharmaceutical formulation comprising interferon-beta and a polyol, a non-reducing sugar or an amino acid, as stabiliser.

Preferably the stabiliser is selected from mannitol, saccharose and glycine; more preferably, the stabiliser is mannitol.

Preferably the liquid pharmaceutical formulation comprises a buffer with a pH between 3 and 4; more preferably, acetate buffer.

Another object of this invention is to provide a process for the preparation of such liquid pharmaceutical formulation comprising the stage of dilution of IFN-β with a solution of the excipients.

Yet another object of the present invention is to provide a presentation form of the liquid pharmaceutical formulation comprising the previously mentioned formulation, hermetically sealed under sterile conditions in a container suitable for storage prior to use.

To study the stability of liquid formulations of IFN-β, various formulations were prepared diluting bulk IFN-β in different buffers at varying pH, then storing the samples at different temperatures and carrying out assays with the immunological test at set intervals of time. Once the buffer solution and the preferred pH, with which the greater stability is obtained, have been selected, then the stabilised formulations of the invention are prepared by diluting the interferon bulk solution with the buffer solution containing also the excipients. Stability of the various formulations was determined by measuring the residual activity of IFN-β at

fixed intervals of time, after storage of the solution at the temperatures of 50°C, 37°C, and 25°C.

To determine such activity, samples were assayed under immunological and biological tests.

The immunological test was carried out by using the TORAY kit (Human IFN-Beta ELISA Kit, TORAY INDUSTRIES, Inc.), following the methodology reported in the enclosed instructions.

The biological dosage was performed as described by Armstrong J.A. (1981), Cytopathic effect inhibition assay for Interferon, in Methods in Enzymology 73 381-387. This test permits the measuring of IFN-β activity by exploiting its anti-viral capacity.

Measure of activity is expressed in International Units per millilitre of solution (IU/ml) or in Mega International Units per millilitre of solution (MIU/ml). (1 MIU/ml = 1,000,000 IU/ml).

An International Unit is calculated as described in the Research Reference Reagent Note No. 35, published by the National Institute of Health, Bethesda, Maryland, in relation to the HuIFN-beta NIH Reference Reagent Gb 23-902-531 used as standard.

The measurement is reported here as percentage of residual activity of the sample of Interferon-beta in the various formulations, taking activity of the sample at time zero as equal to 100%.

Dosages were carried out in duplicate.

To assess the effect of the pH on stability of the active ingredient, different formulations of recombinant IFN-β were prepared containing 0.6 and 1 MIU/ml with various buffer solutions, i.e. acetate buffer, citrate buffer, ascorbate buffer, succinate buffer.

The formulations containing recombinant IFN-β with the buffer solutions were prepared and stored at temperatures of 50°C, 37°C and 25°C, then assayed under the immunological test at set time intervals. The formulations were prepared in such a way as to have a pH between 3.0 and 4.0 and between 5.0 and 6.0, all with buffer at a concentration of 0.01 M.

Tables 1, 2 and 3 report results of tests carried out at set intervals of time, from 1 to 42 days, at the various temperatures.

Data contained in the above-mentioned tables indicate that the formulations with a pH between 5.0 and 6.0 show an immediate loss of